Patent Claims

1. A method for the detection of cytosine methylation in DNA samples, characterized in that the following steps are conducted:

   a genomic DNA sample, which comprises the DNA to be investigated as well as background DNA is treated with bisulfite (= disulfite, hydrogen sulfite) in such a way that all of the unmethylated cytosine bases are converted to uracil, while the 5-methylcytosine bases remain unchanged;

   the bisulfite treated DNA sample is amplified with the use of at least 2 primer oligonucleotides as well as a polymerase, wherein the DNA to be investigated is preferred over the background DNA as the template and

   the amplification is conducted in the presence of at least one additional oligonucleotide or a PNA oligomer, which binds to a 5'-CG-3' dinucleotide or a 5'-TG-3' dinucleotide or a 5'-CA-3'-dinucleotide, whereby the other oligonucleotide or PNA oligomer preferably binds to the background DNA and adversely affects its amplificationperformed

   and the methylation status in the DNA to be investigated is concluded from the presence and /or the amount of the amplified products and/or from the analysis of additional positions.

2. The method of claim 1 wherein a control fragment is amplified simultaneously to the amplification of the bisulfite treated DNA within the same reaction mixture.

3. The method of claim 1, characterized in that the DNA to be investigated comprises GSTP1 or its regulatory region.

4. The method according to claim 3 characterized by and employing thereby at least one primer out of the group consisting of SEQ ID NOs: 2, 42, 54, 58, 62 and 66 and one primer out of the group consisting of SEQ ID NOs: 3, 36, 38, 40, 43, 56, 60, 64 and 68 and at least one blocker out of the group of SEQ ID NO 4, 46, 48, 50, 52, 70, 72, 74,

76, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 102, 103 and SEQ ID NO:104 is used.

5. The method according to claim 3, characterized in that at least one primer oligonucleotide from the group consisting of SEQ ID NOs: 2, 42, 54, 58, 62 and 66 and one primer oligonucleotide from the group SEQ ID NOs: 3, 36, 38, 40, 43, 56 and one additional oligonucleotide from the group consisting of SEQ ID NOs 4, 46, 48, 50, 52, 70, 72, 74 and 76 is used.

6. The method according to one of the preceeding claims, further characterized in that the sample DNA is obtained from serum or urine or other bodily fluids of an individual.

7. The method according to claim 1-5, further characterized in that the DNA samples are obtained from cell lines, blood, sputum, stool, urine, serum, cerebro-spinal fluid, tissue embedded in paraffin, for example, tissue from eyes, intestine, kidneys, brain, heart, prostate, lungs, breast or liver, histological slides and all possible combinations thereof.

8. The method according to one of the proceeding claims, further characterized in that said additional oligonucleotide is a ribonucleic acid oligonucleotide.

9. The method according to one of the preceeding claims, further characterized in that the binding site of the additional oligonucleotide or PNA oligomer overlaps with the binding site of one of the primers on the background DNA and hereby hinders the binding of at least one primer oligonucleotide to the background DNA.

10. The method according to one of the preceeding claims, further characterized in that the additional oligonucleotides and/or PNA oligomers are present in at least five times the concentration in comparison to the primer oligonucleotides.

11. The method according to one of the preceeding claims, further characterized in that the additional oligonucleotides and/or PNA oligomers bind to the background DNA

and thus hinder the complete elongation of primer oligonucleotides in the polymerase reaction.

12. The method according to one of the preceding claims, further characterized in that the chemically treated DNA sample is amplified in the second step with the use of at least 2 primer oligonucleotides and one additional oligonucleotide or PNA oligomer, which hybridizes to a 5'-CG-3' dinucleotide or a 5'-TG-3' dinucleotide or a 5'-CA-3' dinucleotide, and at least one reporter oligonucleotide, which hybridizes to a 5'-CG-3' dinucleotide or a 5'-TG-3'dinucleotide or a 5'-CA-3' dinucleotide, as well as a polymerase; whereby the additional oligonucleotide or PNA oligomer preferably binds to the background DNA and adversely affects its amplification, and whereby the reporter oligonucleotide preferably binds to the DNA to be investigated and indicates its amplification.

13. The method according to one of the preceeding claims, further characterized in that different reporter oligonucleotides indicate the amplification of different products amplified simultaneously in one vessel.

14. The method according to one of the preceeding claims further characterized in that the additional reporter oligonucleotide indicates the presence of the control fragment.

15. The method according to one of the preceeding claims, further characterized in that the reporter oligonucleotides are LightCycler probes, and a LightCycler assay is conducted.

16. The method according to one of the preceeding claims, further characterized in that the reporter oligonucleotides by bearing at least one fluorescent label indicate the amplification either by an increase or a decrease in the fluorescence.

17. The method according to one of the preceding claims, further characterized in that the background DNA is present in 4000 times the concentration of the DNA to be investigated.

18. The method according to one of the preceding claims, further characterized in that the background DNA is present in 8000 times the concentration of the DNA to be investigated.

19. The method according to one of the preceding claims, characterized in that the control fragment is located within the same genomic region of the DNA to be investigated.

20. The method according to one of the preceeding claims, further characterized in that the control fragment is located within a region of 2 kb upstream or 2 kb downstream of the CpG sites analysed.

21. The method according to one of the preceeding claims, further characterized in that the control fragment is located within a region of maximum 1 kb upstream or maximum 1 kb downstream of the CpG sites analysed.

22. The method according to one of the preceeding claims, characterized in that the genomic DNA to be investigated is the GSTP1 gene.

23. The method according to one of the preceeding claims, characterized in that the genomic DNA to be investigated lies within bp 1183 and bp 1309 of the sequence defined by the Genbank Accession Number M24485.1.

24. The method according to one of the preceeding claims, characterized in that the genomic DNA to be investigated lies within bp 1183 and bp 1309 of the sequence defined by the Genbank AccessionNumber M24485.1 and the control fragment lies within bp 2273 and bp 2303 of the sequence defined by the Genbank Accession Number M24485.1

25. A kit comprising at least one primer oligo nucleotide out of the group consisting of SEQ ID NOs: 2, 42, 54, 58, 62 and 66 and one primer (reverse) out of the group consisting of SEQ ID NOs: 3, 36, 38, 40, 43, 56, 60, 64 and 68 and at least one blocker out of the group of SEQ ID NO 4, 46, 48, 50, 52, 70, 72, 74, 76, 80, 81, 82,

83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 102, 103 and SEQ ID NO:104.